Received: 25 June 2020 | Revised: 10 November 2020 | Accepted: 20 January 2021

DOI: 10.1002/ppp3.10185

RESEARCH ARTICLE

Plants People Planet PPP Open Access

Specific arbuscular mycorrhizal fungal–plant interactions determine radionuclide and metal transfer into *Plantago lanceolata*

Jeanette Rosas-Moreno | **Jon K. Pittman** | **Clare H. Robinson**

Department of Earth and Environmental Sciences, School of Natural Sciences, The University of Manchester, Manchester, UK

Correspondence
Jon K. Pittman, Department of Earth and Environmental Sciences, School of Natural Sciences, The University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK.
Email: jon.pittman@manchester.ac.uk

Funding information
CONACyT; NERC, Grant/Award Number: NE/L000547/1

Societal Impact Statement

Industrial activity has left a legacy of pollution by radionuclides and heavy metals. The exposure of terrestrial environments to increased levels of ionising radiation and toxic elements is of concern, not only because of the immediate effects to biota but also because of the potential risk of mobilisation into higher levels of a food chain. Here, we present a study that extends our knowledge of how arbuscular mycorrhizal fungi contribute to the mobilisation of non-essential elements in environments such as former mine sites, and provides a perspective that will be of interest for the management and remediation of such sites.

Summary

- Accumulation and transfer of long-lived radionuclides and toxic metals in terrestrial environments is of major concern because of potential mobilisation into food chains. In this study, we aimed to compare the role of four different arbuscular mycorrhizal fungal (AMF) cultures on the transfer of non-essential elements into *Plantago lanceolata* from a naturally contaminated soil source.
- Soil from an abandoned uranium (^{238}U) mine was collected as a natural source of ^{238}U, thorium (^{232}Th), arsenic (As) and lead (Pb). *P. lanceolata* was inoculated with four AMF cultures (*Rhizophagus irregularis* DAOM181602, *Acaulospora longula* BEG8, *Scutellospora calospora* BEG245 and *Funneliformis mosseae* BEG12) to compare the uptake and transfer from root to shoot. Inductively coupled plasma (ICP) mass spectroscopy and ICP-absorption emission spectroscopy analyses provided quantification of total elemental concentrations in soil and plant tissues.
- Two of the AMF cultures, *A. longula* and *F. mosseae*, had contrasting roles in toxic element partitioning in plant tissue of *P. lanceolata*. *F. mosseae* increased the accumulation of ^{238}U, ^{232}Th, Pb, As and Cu in shoots whereas *A. longula* induced increased partitioning of ^{232}Th, Ca, Fe and Zn in roots. The inoculation treatments and the differential accumulation of these elements had no significant effect on plant biomass.
- The use of different AMF cultures in enhancing phytoremediation of contaminated environments requires a wider understanding of the contribution of different AMF cultures to non-essential element acquisition as well as to plant nutrition.

KEYWORDS
arbuscular mycorrhizal fungi, arsenic, lead, *Plantago lanceolata*, radionuclides, thorium, uranium

1 | INTRODUCTION

Understanding the mechanisms of transfer and bioaccumulation of inorganic pollutants such as non-essential metals and radionuclides into biota is important to assess the toxicological risks of these elements to the environment and to human health. Anthropogenic activities such as mining and agri-chemical usage have led to greater exposure in natural environments to potentially toxic metals and metalloids, such as lead (Pb) and arsenic (As), and radionuclides including uranium (U) and thorium (Th). In terrestrial ecosystems, plants are primary producers and are therefore an entry point for toxins within an ecosystem (Declerck et al., 2003). Methods to determine the risks of trophic transfer of such elements typically involve simple measurements from field samples but often lack an integrated analysis of the path that elements follow from soil into the trophic chain (Beresford et al., 2008; Willey, 2014).

^{238}U and ^{232}Th are two of the most common isotopes occurring in the Earth's crust. They have no known biological function but accumulation within organisms can be damaging due to radioactivity and chemotoxic effects (Saenen et al., 2013). In uncontaminated terrestrial environments, U and Th concentration in plants is low, while in environments with elevated concentrations (either naturally or by human activities) plants are able to accumulate radionuclides into their cells (Davies et al., 2018; Shtangeeva, 2010). Investigations of radionuclide transfer into plants often ignore the role that soil microbiota has on this process despite the fundamental symbiotic interaction between many plant species and arbuscular mycorrhizal fungi (AMF; Davies et al., 2015). AMF (Glomeromycota; Redecker et al., 2013—also classified as Glomeromycotina; Spatafora et al., 2016) colonise most land plants and can facilitate the transfer of essential nutrients, such as phosphorus (P), allowing plants to be competitive in their environment (Schüßler & Walker, 2011; Smith & Read, 2008). While many previous studies have examined the roles of AMF on plant nutrient acquisition and growth improvement, more recent studies have considered the functional diversity of AMF, their influence on plant communities and host stress tolerance, including the possibility of providing protection against toxic element accumulation (Helgason et al., 2002; Sánchez-Castro et al., 2017; van der Heijden et al., 2014).

Previous studies have indicated that AMF associations, especially with *Rhizophagus irregularis*, can lead to increased concentration of U in root tissues, and may contribute to phytostabilisation of U by reducing concentration within the root symplast and restricting uptake into plant shoots (Chen et al., 2008; Rufyikiri et al., 2004). This suppression of shoot transfer may be due to U sequestration into AMF vesicles and spores (Weiersbye et al., 1999). However, it is unclear exactly how important AMF association is in suppressing U bioaccumulation in contrast with abiotic factors; for example, translocation of U is strongly dependent on soil pH conditions (Rufyikiri et al., 2002), while increased soil P availability can reduce U translocation into plant shoots (Chen, Jakobsen, et al., 2005). Therefore, further examination into the potential role of AMF as a pathway for non-essential element transfer into plants is needed.

The aim of this study was to examine the transfer of naturally occurring radionuclides (^{238}U and ^{232}Th) as well as other non-essential elements (As and Pb) into plants via the action of AMF species, and assess these in comparison to essential elements. The hypothesis was that AMF would significantly influence the transfer of non-essential elements into the host plant but that different fungal cultures would elicit different responses. We have made use of *Plantago lanceolata* as a host plant, and four AMF species: *R. irregularis*, a commonly used 'model' AMF, and *Acaulospora longula*, *Funneliformis mosseae* and *Scutellospora calospora*, which are found in UK locations and thus relevant to this study. We used natural soil from a U mine site containing elevated concentrations of ^{238}U and ^{232}Th as well as other essential and non-essential elements (Davies et al., 2018).

2 | MATERIALS AND METHODS

2.1 | Plant material and AMF culture propagation

Plantago lanceolata seeds (Emorsgate Seeds) were surface sterilised in 10% (v/v) sodium hypochlorite, 0.5% (v/v) Triton-X100 for 10 min followed by rinsing in sterile Milli-Q water (Millipore) before being sown. Seeds were left to germinate and grow for 4 weeks in 1:1 (v/v) autoclaved mixture of Terra Green (oil-dri.co.uk) and lime-free washed fine grade silica sand (Royal Horticultural Society). Plants were grown in a Panasonic versatile environmental chamber (MLR-352-PE) set to 40 µmol m^{-2} s^{-1} illumination, 16 hr light at 22°C and 8 hr dark at 15°C.

The following AMF cultures were used: *R. irregularis* (strain DAOM181602/DAOM197198/MUCL43194; Stockinger et al., 2009) was obtained from SYMPLANTA as a spore-based, ready to use product mixed within a diatomite powder carrier material. *A. longula* (strain BEG8), *F. mosseae* (strain BEG12) and *S. calospora* (strain BEG245) were obtained from the International Bank for the Glomeromycota and are collectively referred to as 'BEG cultures'. Each of the BEG cultures were propagated and maintained in a 1.5 L pot containing a *P. lanceolata* plant in 1:1 Terra Green/sand mixture, and were grown for 6 months in the growth conditions as described above.

2.2 | Preparation of the two-compartmental growth system

The radionuclide and metal contaminated soil was collected in September 2017 from the ore processing area of the disused South

Terras U mine site in Cornwall, UK. The general soil characteristics and conditions of the site were described in Davies et al. (2018); mean soil pH was 4.8, and there was higher than average (for a typical UK sandy soil) concentration of ^{238}U, ^{232}Th, As and Pb (Table 1). South Terras soil was sieved to remove large particles and stones. The non-contaminated, low nutrient (Table 1) Henfaes sandy soil was collected in April 2015 from the Henfaes Research Centre, Bangor, UK. Six small sub-samples of each soil were taken for detailed chemical analysis (see Section 2.4) while the rest of the soil was used for preparing the two-compartment system. Both sets of soil were sterilised by gamma irradiation at 30–45 kGy at the Dalton Cumbrian Facility, University of Manchester, UK. Fine grade silica sand was autoclaved twice for 1 hr at 130°C with at least 1 day rest period between cycles.

An experimental exposure and growth system composed of Compartment A (CA) and Compartment B (CB) was modified from previous designs by Smith (2003) and Chen, Jakobsen, et al. (2005; Figure 1). CB consisted of a 75 ml polypropylene vial containing 50 g of South Terras soil with 20 g of autoclaved Terra Green placed on top as a buffer zone. The vial was sealed with nylon mesh (Sefar Ltd), either a coarse mesh (CM) of 700 μm pore size or a fine mesh (FM) of 35 μm pore size. Parafilm (Sigma Aldrich) was used to fix the mesh to the vials. CB was placed with the mesh on one side within CA, which consisted of a non-draining 1 L plastic pot containing a 2:1 (v/v) mixture of silica sand and Henfaes sandy soil.

Two 4-week-old *P. lanceolata* seedlings were transplanted into each container, directly into CA. For the *R. irregularis* treatments, 5 g of inoculum containing spores were mixed into the CA substrate. The reciprocal non-mycorrhizal (control-1) treatment received no *R. irregularis* inoculum but did receive the equivalent amount of carrier material used for the inoculum. For the BEG culture treatments, 60 g of previously prepared inoculum (see Section 2.1), which included root fragments, spores and mycelium were mixed into the CA substrate. The BEG culture treatments included a separate non-mycorrhizal (control-2) treatment. For each treatment there was a FM version and a CM version, and there were six replicates per treatment, and so in total there were 12 *R. irregularis* inoculated pots, 12 control-1 pots, 12 pots for each BEG culture and 12 control-2 pots (72 pots overall). All treatments were incubated under controlled conditions as described in Section 2.1. Pots were randomly distributed in the growth chamber and rotated weekly. Watering was carried out weekly by weighing the pots and maintaining the water holding capacity at approximately 55%.

2.3 | Harvest and sample preparation

Plants inoculated with *R. irregularis* (and from control-1 pots) were harvested after 8 weeks and plants inoculated with BEG cultures (and from control-2 pots) were harvested after 12 weeks, because preliminary experiments showed that BEG culture association with *P. lanceolata* was slower. During harvest, plants and CB vials were carefully removed from CA. Roots and shoots were separated with a scalpel blade, rinsed with Milli-Q water, and dried with tissue paper. All shoot and root tissues were kept for chemical analysis. A small portion of root tissue from FM treatments was retained for AMF staining. Root tissue from CM treatments was distinguished between root tissue that had not entered CB and root tissue that had entered CB. These samples were weighed separately while a small portion was retained for staining.

2.4 | Chemical analysis

In preparation for acid digestion, all shoot and root tissue samples, and soil sub-samples were dried at 70°C for 48 hr. Dry weight measurements were recorded. Dried soil was ground by mortar and pestle then 0.1 g of soil was placed in borosilicate tubes and incubated in 5 ml of analytical grade 70% (v/v) nitric acid at 140°C for 3 hr. Dried plant samples (0.1 g) were incubated overnight in 5 ml

TABLE 1 Chemical composition of South Terras contaminated soil and Henfaes low phosphate sandy soil

Non-essential elements		Macronutrients		Micronutrients	
South Terras contaminated soil					
^{238}U	479.29 μg/g (*SD* = 77.71)	P	1.36 mg/g (*SD* = 0.11)	Cu	0.86 mg/g (*SD* = 0.21)
^{232}Th	1.33 μg/g (*SD* = 0.13)	K	0.99 mg/g (*SD* = 0.05)	Fe	59.44 mg/g (*SD* = 3.42)
As	1.79 mg/g (*SD* = 1.76)	Ca	4.8 mg/g (*SD* = 0.23)	Mn	0.45 mg/g (*SD* = 0.02)
Pb	561.35 μg/g (*SD* = 39.73)	Mg	1.15 mg/g (*SD* = 0.6)	Zn	0.29 mg/g (*SD* = 0.03)
		S	2.01 mg/g (*SD* = 0.23)		
Henfaes low phosphate sandy soil					
^{238}U	0.01 μg/g (*SD* = 0.01)	P	0.24 mg/g (*SD* = 0.06)	Cu	2.15 μg/g (*SD* = 0.57)
^{232}Th	1.46 μg/g (*SD* = 0.29)	K	0.42 mg/g (*SD* = 0.11)	Fe	3.92 mg/g (*SD* = 1.12)
As	1.67 μg/g (*SD* = 0.65)	Ca	0.08 mg/g (*SD* = 0.03)	Mn	0.09 mg/g (*SD* = 0.03)
Pb	5.12 μg/g (*SD* = 1.84)	Mg	1.51 mg/g (*SD* = 0.8)	Zn	0.03 mg/g (*SD* = 0.01)
		S	Not determined		

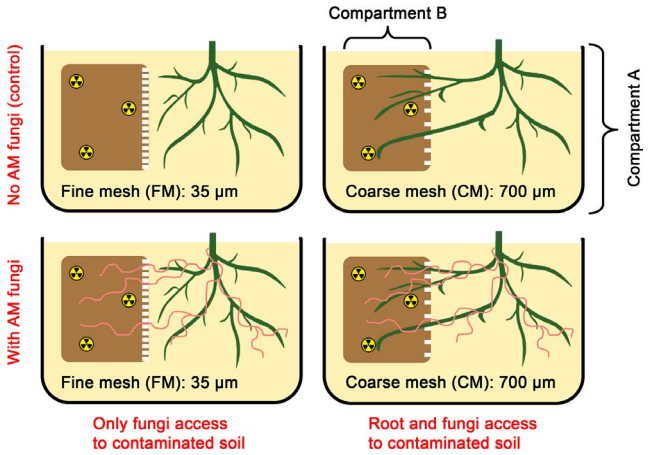

FIGURE 1 Design of the two-compartment cultivation system. Compartment A (CA) is a pot containing plants in low-phosphate sandy soil and Compartment B (CB) is a vial containing contaminated soil from South Terras mine. CB vials are separated from CA with either a fine mesh (FM) or a coarse mesh (CM). Roots are shown in green and hyphae in red. There are four possible scenarios: a control treatment without arbuscular mycorrhizal fungal (AMF) inoculation where roots cannot access CB (top left); a control treatment without AMF inoculation where roots can access CB (top right); a treatment with AMF inoculation where only hyphae can access CB (bottom left); a treatment with AMF inoculation where hyphae and roots can access CB (bottom right)

of analytical grade 70% (v/v) nitric acid then microwaved in TFM vessels in a MARS5 digestion oven. Cooled samples were diluted in Milli-Q water and filtered through a 0.45 µm Millipore MCE filter before analysis by inductively coupled plasma mass spectroscopy (ICP-MS) or inductively coupled plasma absorption emission spectroscopy (ICP-AES), as appropriate. Quantification of As, Pb, Th, Ti and U was determined by ICP-MS using an Agilent 7700x (Agilent) fitted with a collision cell, pressurised with He at a flow rate of 4.5 ml/min. Quantification of Ca, Cu, Fe, K, Mg, Mn, P, S and Zn was determined by ICP-AES using a Perkin-Elmer Optima 5,300 (Perkin-Elmer). Certified Reference Standard TM25.5 was used for all ICP analyses. Plant root element concentrations reported from ICP analysis were adjusted with a Ti correction factor (Ti%) to account of the soil particles attached to plant tissue exactly as described previously (Davies et al., 2018).

2.5 | AMF staining and quantification

The root preparation and staining procedure was as described by Koske and Gemma (1989) and Walker (2005). The estimation of the proportion AMF structures colonising roots was carried out using the intersection method (McGonigle et al., 1990), which records the presence and absence of arbuscules and hyphae per intersection. This was performed as described previously (Davies et al., 2018) except that micrographs were taken under bright field microscopy with a GXM-L2800 High Power Microscope and a camera model GXCAM-U3.

2.6 | Statistical analysis

Statistical analyses of data were performed using JMP and Origin statistical software and graphed using GraphPad PRISM. Two-Way ANOVA was performed to examine the interactive effect of AMF treatment, either between control-1 and *R. irregularis* inoculated plant data only (Table S1) or control-2 and BEG culture inoculated plant data only (Table S2), and mesh size. Multiple comparisons were performed from the separate 2 level and 4 level ANOVA using Tukey's multiple comparison post-hoc test to determine significant ($p < .05$) differences between treatments. Principal component analysis (PCA) was performed using a matrix of 72 samples (6 replicates from 12 treatments including all AMF treatments, both control sets, both mesh size treatments) with 13 variables (tissue concentration of As, Pb, Th, U, Ca, K, Mg, P, S, Cu, Fe, Mn and Zn) for shoot and root tissue separately. All input data were natural log transformed to account for order of magnitude variation in concentration between some elements).

3 | RESULTS

3.1 | Effect of AMF colonisation on *P. lanceolata* biomass

Arbuscular and hyphal colonisation of *P. lanceolata* following *R. irregularis* inoculation was not significantly different between CM and FM treatment conditions after 8 weeks of growth, and neither were there significant differences in AMF colonisation between CM and FM treatments for each of the BEG cultures (*A. longula* BEG8, *F. mosseae* BEG12 and *S. calospora* BEG245), and among the BEG cultures after 12 weeks of growth (Figures S1–S6). No AMF structures or any other fungal structures including other endophytes were observed in the roots of non-inoculated control plants.

Shoot dry weight of plants inoculated with *R. irregularis* was significantly lower compared with the control in the CM treatment in which both roots and fungal hyphae were able to access the nutrient-rich South Terras soil compartment, although there was no significant difference in the FM treatment where only fungal hyphae could access the South Terras soil (Figure 2a). In contrast, root dry weight of the inoculated FM treatment, but not the CM treatment was significantly lower than the control (Figure 2c). Moreover, there was significantly higher production of root mass in the control FM plants than the control CM plants, indicating that plants without access to nutrient rich soil and without mycorrhizal association initially had enhanced root growth, although only up to 12 weeks of growth (Figure 2d). There were no significant differences in shoot dry weight between inoculated and control plants for any of the BEG cultures, although the *A. longula* FM treatment was significantly lower than the *F. mosseae* FM treatment (Figure 2b). Only root dry weight of the *S. calospora* CM treatment was significantly lower compared to the control but there were no differences between FM treatments on the basis of root growth for the BEG cultures (Figure 2d).

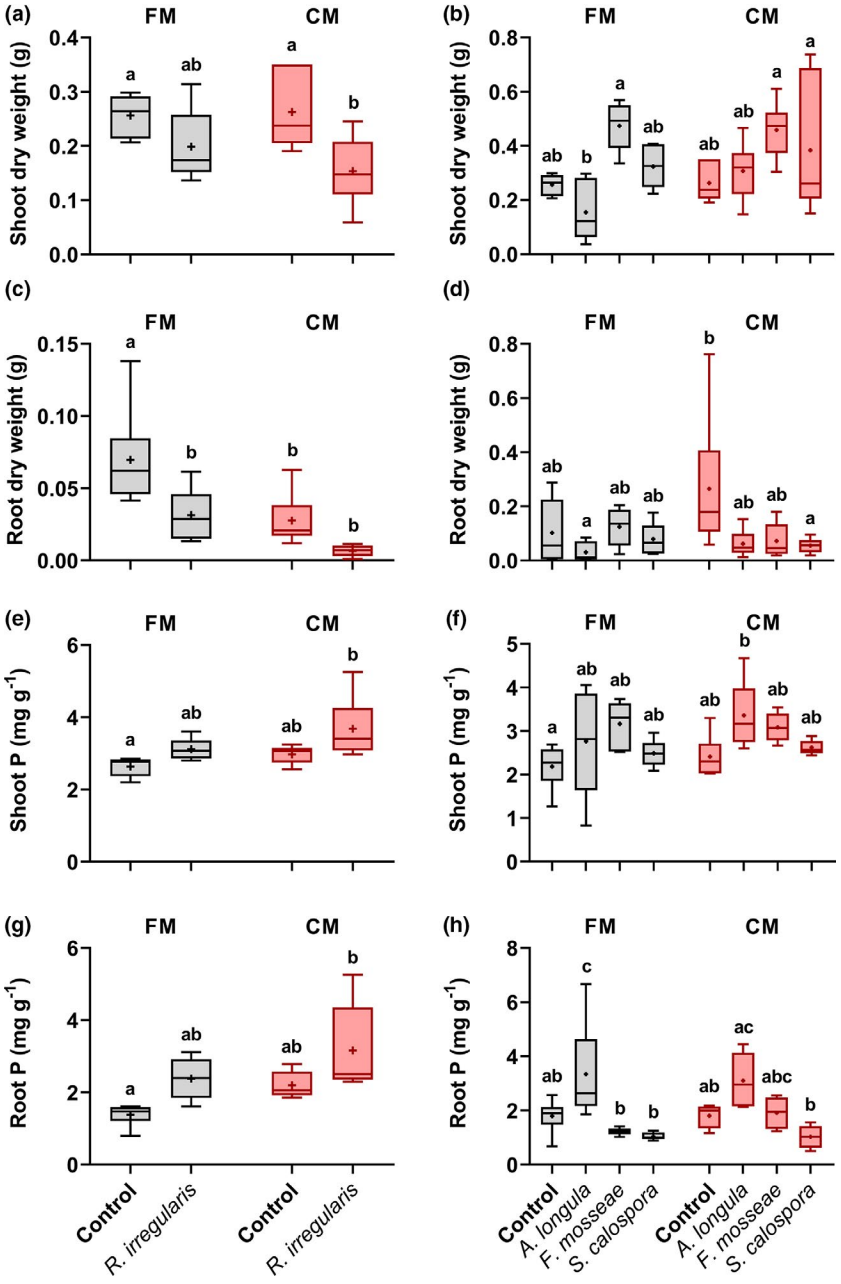

FIGURE 2 Dry weight shoot (a,b) and root (c,d) biomass, and shoot (e,f) and root (g,h) P concentration of *Plantago lanceolata* inoculated with *Rhizophagus irregularis* in comparison to non-inoculated control plants after 8 weeks growth (a,c,e,g), and with BEG cultures (*Acaulospora longula*, *Funneliformis mosseae*, and *Scutellospora calospora*) in comparison to non-inoculated control plants after 12 weeks growth (b,d,f,h). Coarse mesh (CM) and fine mesh (FM) denotes coarse mesh and fine mesh treatments, respectively. Boxes show the 25th and 75th percentile values, the black line within the boxes shows the median value, the cross shows the mean value and whisker bars show the minimum and maximum values. Values that do not share lowercase letters are significantly different ($p < .05$)

3.2 | Effect of AMF colonisation on radionuclide and heavy metal accumulation into *P. lanceolata*

When the *P. lanceolata* roots were unable to access the contaminated South Terras soil due to the presence of the FM, the concentrations of non-essential elements in plant tissues were low, in comparison to average concentrations for plants grown directly in this soil (Davies et al., 2018). After 8 weeks growth, even when the roots could access the contaminated soil (control CM vs. control FM) there was no increase in root concentrations of ^{238}U, ^{232}Th, As or Pb, and only shoot concentration of As were increased (Figure 3). Furthermore, inoculation with *R. irregularis* had no effect on the concentration of these elements apart from increasing As to detectable levels in the shoots of FM treatment plants. In contrast, after 12 weeks growth and with greater root mass, the CM plants appeared to have more efficient access the South Terras soil and showed significantly increased ^{238}U and As concentration in the roots (control CM vs. control FM, Figure 4b,f), and indeed much higher tissue concentration of As compared to the 8 week old plants (Figure 3f vs. 4f), although there was no significant difference in shoot concentrations of these elements (Figure 4a,e). However, for the FM treated plants, the addition of any of the BEG cultures did not increase root concentration of ^{238}U and As. The addition of *F. mosseae* significantly reduced root ^{238}U concentration in the CM treatment (Figure 4b) but increased shoot ^{238}U concentration in the FM treatment (Figure 4a). The presence of this AMF culture was likewise able to reduce Pb concentration in the CM treatment roots (Figure 4h) and increase Pb and As concentration in the FM treatment shoots (Figure 4e,g). Furthermore, shoot ^{232}Th concentration was high in the FM plants inoculated with *F. mosseae* (Figure 4c). *A. longula* was the only culture

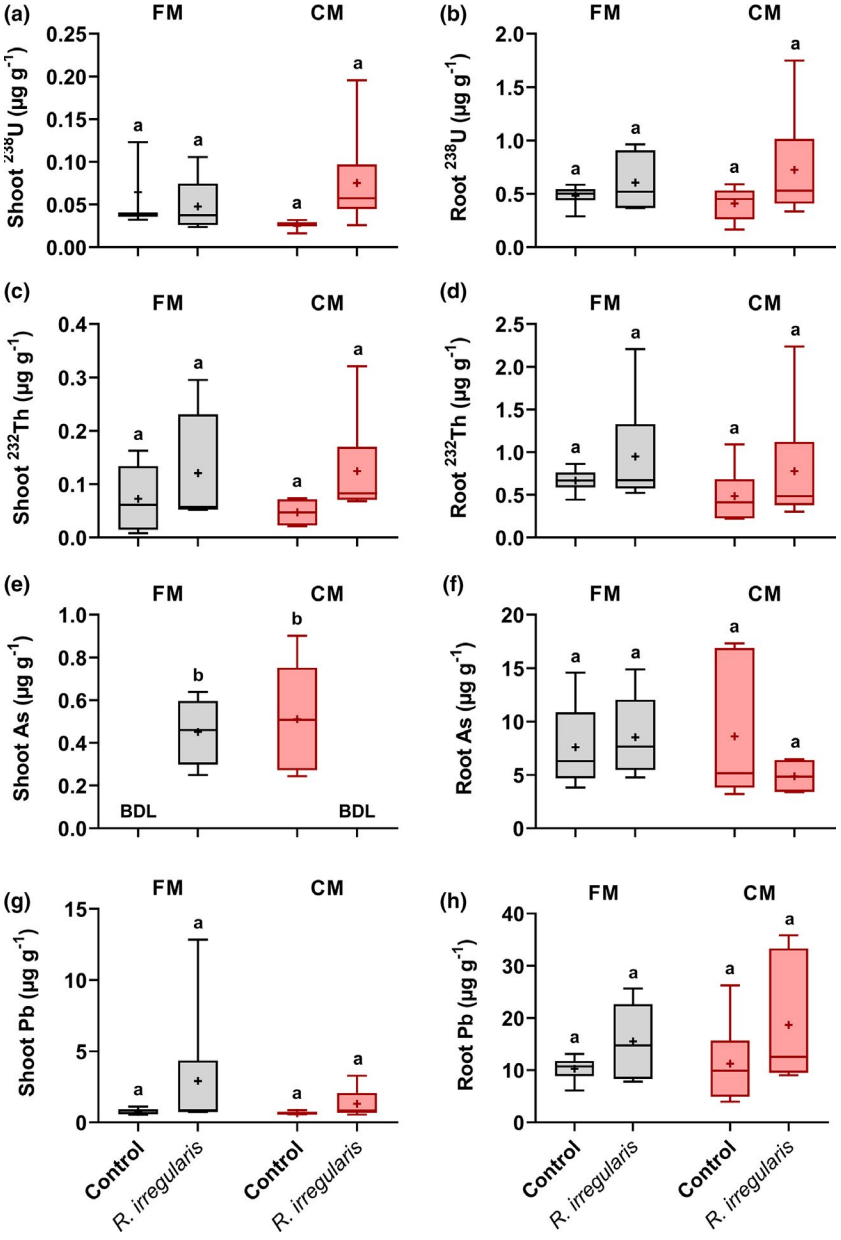

FIGURE 3 Concentrations of non-essential elements U, Th, As and Pb in shoots (a,c,e,g) and roots (b,d,f,h) of *Plantago lanceolata* inoculated with *Rhizophagus irregularis* in comparison to non-inoculated control treatments after 8 weeks growth. Coarse mesh (CM) and fine mesh (FM) denotes coarse mesh and fine mesh treatments, respectively. Boxes show the 25th and 75th percentile values, the black line within the boxes shows the median value, the cross shows the mean value and whisker bars show the minimum and maximum values. Values that do not share lowercase letters are significantly different ($p < .05$)

that significantly increased ^{232}Th concentration in FM treated roots (Figure 4d) although shoot ^{232}Th content was undetectable.

3.3 | Effect of AMF colonisation on essential nutrient accumulation into *P. lanceolata*

After 8 weeks growth, P concentration in shoots and roots were not significantly different between CM and FM treatments, and there was no difference between the presence and absence of *R. irregularis* (Figure 2). Likewise, inoculation by any of the BEG cultures did not significantly alter the shoot P accumulation (Figure 2f); however, the addition of *A. longula* gave significantly higher P concentration in roots from the FM treatments than all other FM treatments (Figure 2h). *A. longula* CM root P concentration values were significantly higher than the same treatment inoculated with *S. calospora*. Concentrations of calcium (Ca), potassium (K), sulphur (S) and magnesium (Mg) in shoots were unaffected by any AMF treatments, except for the *R. irregularis* FM treatment where K concentration was higher than the FM control (Figure S7). Some of the root macronutrients were affected by fungal inoculation. All BEG cultures induced significantly decreased K concentration for the FM treatments compared with the control (Figure 5). Ca concentration was significantly higher in *A. longula* roots compared to the control for both CM and FM treatments (Figure 5b) while *F. mosseae* inoculation reduced S concentration (Figure 5h).

Micronutrients including manganese (Mn), zinc (Zn), copper (Cu) and iron (Fe) were also examined. Inoculation by *R. irregularis* significantly increased Cu concentration in the roots of the CM treatment plants (Figure S8) but there was no difference in shoots, while *F. mosseae* significantly increased Cu concentration in shoots of both FM and CM plants (Figure 6a). *A. longula* significantly decreased Mn concentration in FM plant shoots, while this fungal culture gave rise

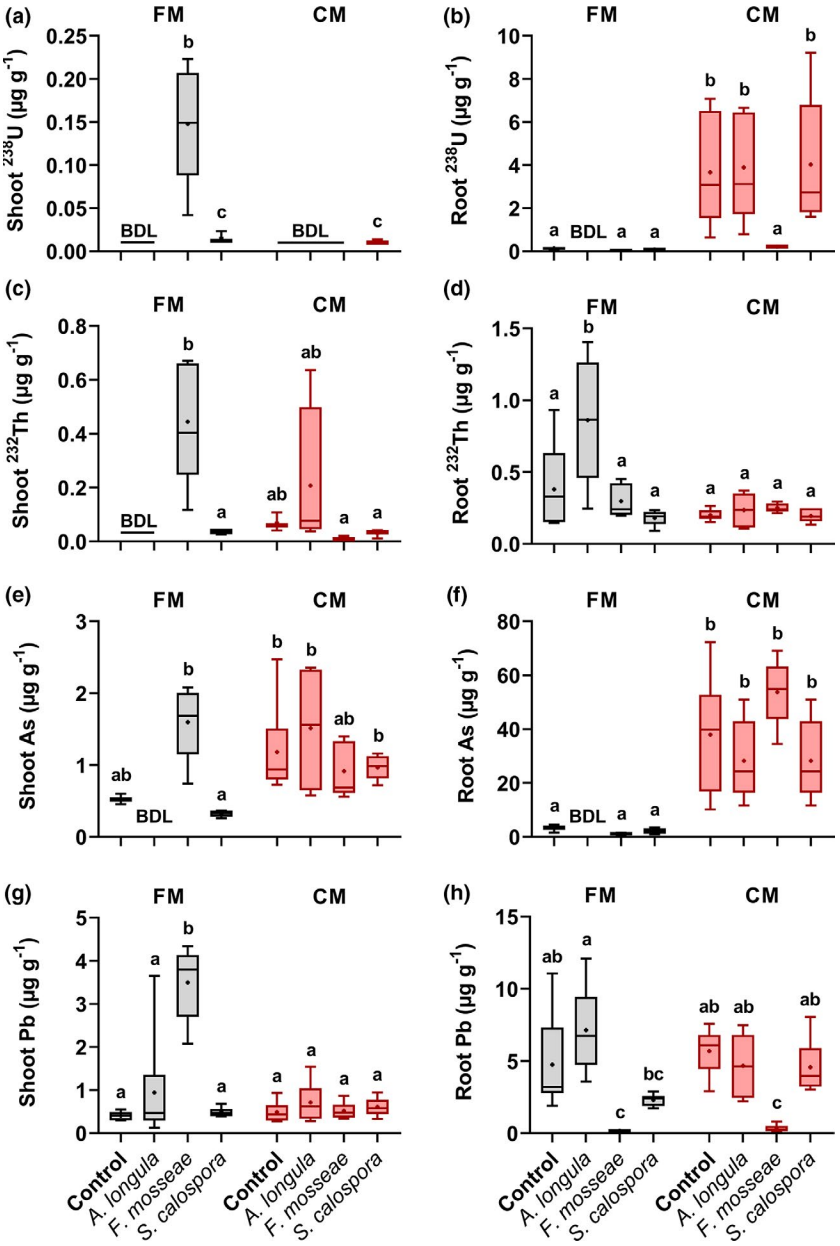

FIGURE 4 Concentrations of non-essential elements U, Th, As and Pb in shoots (a,c,e,g) and roots (b,d,f,h) of *Plantago lanceolata* inoculated with *Acaulospora longula*, *Scutellospora calospora*, and *Funneliformis mosseae* in comparison to non-inoculated control treatments after 12 weeks growth. Coarse mesh (CM) and fine mesh (FM) denotes coarse mesh and fine mesh treatments, respectively. Boxes show the 25th and 75th percentile values, the black line within the boxes shows the median value, the cross shows the mean value and whisker bars show the minimum and maximum values. Values that do not share lowercase letters are significantly different ($p < .05$)

to micronutrient changes within the roots; in particular, there was increased Zn concentration in the FM plants compared to the other inoculation treatments and significantly increased Fe concentration in CM and FM roots (Figure 6).

3.4 | PCA of element accumulation into *P. lanceolata* for all AMF species

To visualise and compare all of the non-essential and essential element changes that were quantified in the plant tissues in response to AMF treatment, PCA was performed for shoot and root tissues separately (Figure 7). For both of the shoot and root biplots, over 40% of the variation in the data was due to principal component 1 (PC1), explained mostly by differences in concentration of essential elements, while variation on the basis of PC2 was explained mostly by differences in concentration of non-essential elements including U and As. There was separation between the two control samples (control-1 for the *R. irregularis* experiment, and control-2 for the BEG cultures experiment), which was especially evident in the shoot tissue data (Figure 7a) and indicated that difference in growth period (8 weeks vs. 12 weeks) causes difference in the essential nutrient and non-essential element accumulation profile as the plant develops. There was greater overlap between AMF treatments within the shoot dataset, particularly with their respective controls. However, there was clear separation between five of the six FM plant samples inoculated with *F. mosseae* on the basis of PC2, and indicative of the enhanced shoot accumulation of U, Th and Pb (Figure 7a). In the root biplot there was clear separation between most of the AMF treatments, particularly for *A. longula* and *F. mosseae* treatments on the basis of both PC1 and PC2 but in opposite directions from the control (Figure 7b). This further indicated the increase in concentration of

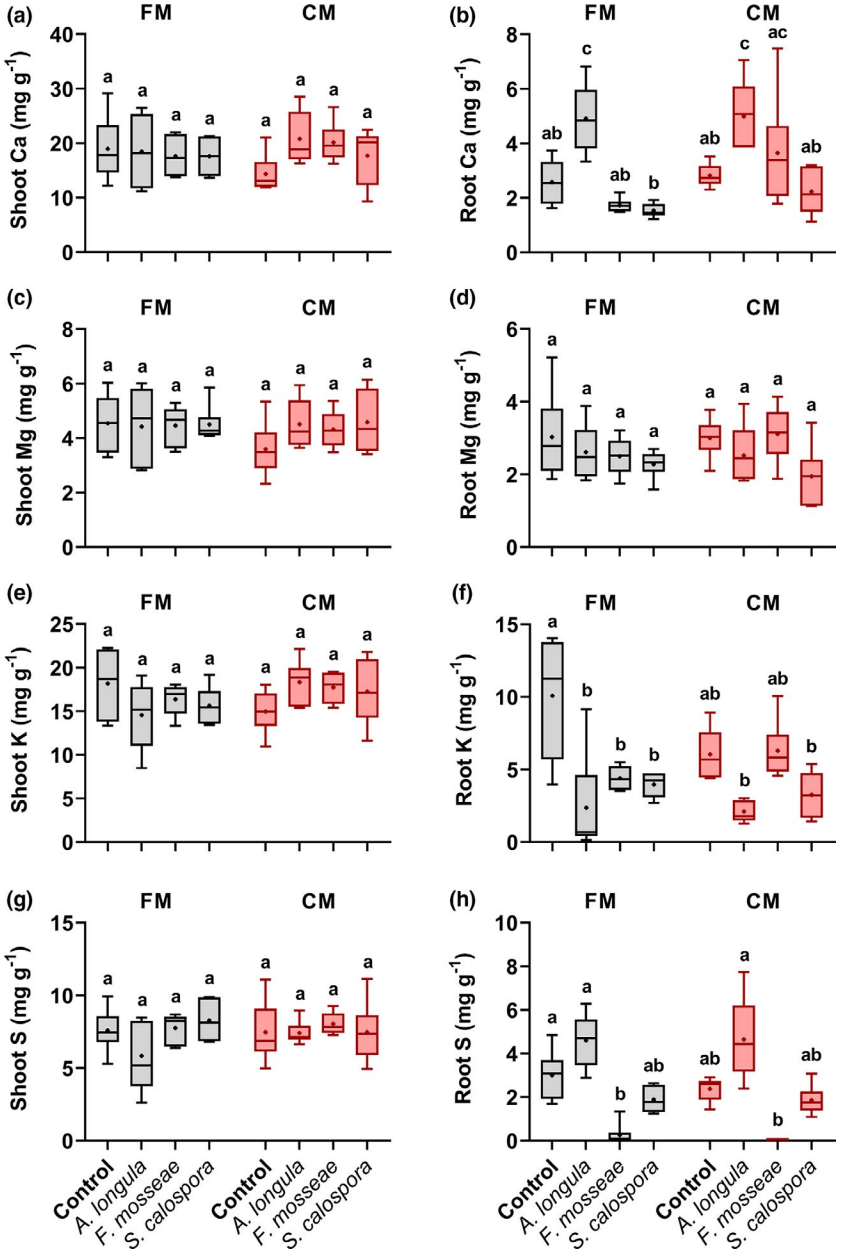

FIGURE 5 Concentrations of essential macronutrients Ca, Mg, K and S in shoots (a,c,e,g) and roots (b,d,f,h) of *Plantago lanceolata* inoculated with *Acaulospora longula*, *Scutellospora calospora*, and *Funneliformis mosseae* in comparison to non-inoculated control treatments after 12 weeks growth. Coarse mesh (CM) and fine mesh (FM) denotes coarse mesh and fine mesh treatments, respectively. Boxes show the 25th and 75th percentile values, the black line within the boxes shows the median value, the cross shows the mean value and whisker bars show the minimum and maximum values. Values that do not share lowercase letters are significantly different ($p < .05$)

multiple elements within *A. longula* roots, including Th and Zn, and the decrease in multiple elements, including Pb, within *F. mosseae* roots.

4 | DISCUSSION

4.1 | Contrasting profiles of radionuclide and metal accumulation induced by specific fungal species

The use of a compartmentalised growth system (Figure 1) has allowed examination of the contribution of AMF to phytoaccumulation and root-to-shoot distribution of elements present in contaminated soil from the South Terras mine site. Care was taken here to sterilise the soil and sand used for plant growth, and although the fungal inoculum used might have contained other microorganisms that could alter element bioavailability, we are confident that this study has predominantly examined the effects of AMF. Many of the plants growing naturally at South Terras are mycorrhizal, including association with *R. irregularis* and *Acaulospora* sp., plus other members of the Glomeraceae family (Davies et al., 2018). However, from our previous field study, it was inconclusive if the presence of AMF explained the patterns of radionuclide and metal transfer into plants. While one-to-one AMF-to-plant interactions are atypical in the natural environment, this approach has allowed us to demonstrate that some fungal cultures can significantly alter plant element distribution but other cultures have no effect or very different effects (Figure 8). Furthermore, it was not simply the presence of AMF association that caused alterations in plant tissue element concentrations but whether access to the soil source was exclusively via AMF, as tested by the use of different mesh sizes. An alternative approach could have been the use of mesh cores that are twisted to severe hyphal connections (Johnson et al., 2001), but we felt that this approach

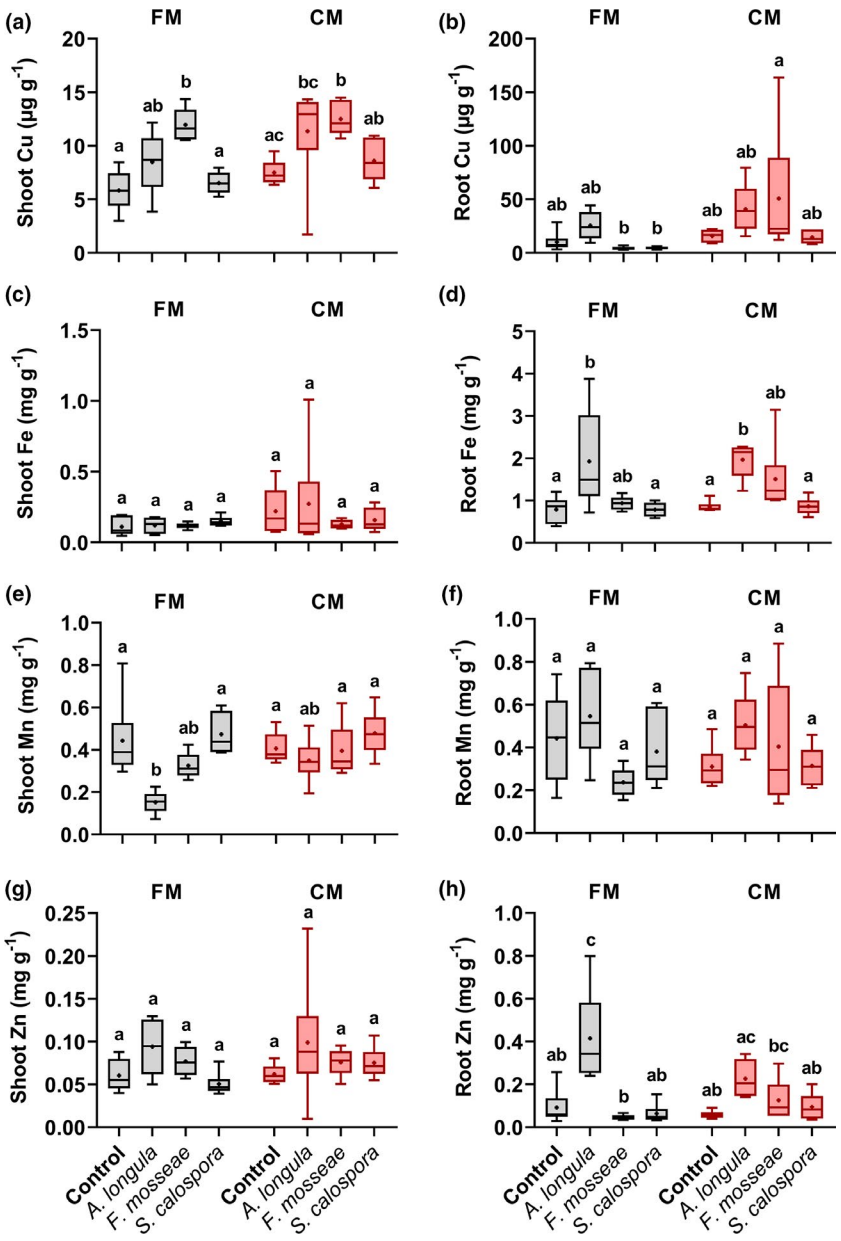

FIGURE 6 Concentrations of essential micronutrients Cu, Fe, Mn and Zn in shoots (a,c,e,g) and roots (b,d,f,h) of *Plantago lanceolata* inoculated with *Acaulospora longula*, *Scutellospora calospora*, and *Funneliformis mosseae* in comparison to non-inoculated control treatments after 12 weeks growth. Coarse mesh (CM) and fine mesh (FM) denotes coarse mesh and fine mesh treatments, respectively. Boxes show the 25th and 75th percentile values, the black line within the boxes shows the median value, the cross shows the mean value and whisker bars show the minimum and maximum values. Values that do not share lowercase letters are significantly different ($p < .05$)

would have been more disruptive. In particular, we found that inoculation with *F. mosseae* increased partitioning of radionuclides (^{238}U and ^{232}Th) and metals (As, Pb, Cu) in shoots when only hyphae were able to access the contaminated soil (Figures 4 and 6). Furthermore, the presence of *F. mosseae* reduced ^{238}U and Pb concentrations in roots. In contrast, there were no equivalent shoot or root changes for any of these elements following *A. longula* and *S. calospora* inoculation, and only As concentration was enhanced in shoots following *R. irregularis* inoculation (Figure 3).

Funneliformis mosseae appears to be efficient at scavenging potentially toxic elements from the soil. When the fern *Pteris vittata* was colonised with *F. mosseae* (=*Glomus mosseae*), As and U accumulated into the roots but with very little translocation to aboveground fronds (Chen et al., 2006). Similarly, *Oryza sativa* associated with *F. mosseae* showed an increased capacity to bind Cu to root cell walls although the total root and shoot Cu concentration was reduced compared to non-mycorrhizal rice, thus reducing toxicity to elevated Cu (Zhang et al., 2009). Similarly, *P. lanceolata* inoculated with *Funneliformis geosporum* had reduced root and shoot As and Pb concentrations (Orłowska et al., 2012). Therefore, while the *F. mosseae*-induced restriction of ^{238}U and Pb in this experiment in *P. lanceolata* roots is similar to root metal restriction observed in other studies, the increase of metals and radionuclides in *F. mosseae*-associated *P. lanceolata* shoots is unusual. However, this did not appear to induce any toxicity and these plants did not display any reduction in root or shoot biomass (Figure 2).

Acaulospora longula also yielded plant responses that were distinct from the other three fungal cultures by inducing increased root concentration of ^{232}Th and various other metals (Ca, Fe, and Zn; Figure 8). In addition, this culture induced higher P accumulation in *P. lanceolata* roots. This could indicate that all or some of these elements are phytoaccumulated via shared pathways. Fe^{2+} and Zn^{2+} transport into extraradical mycelia and subsequent transfer into

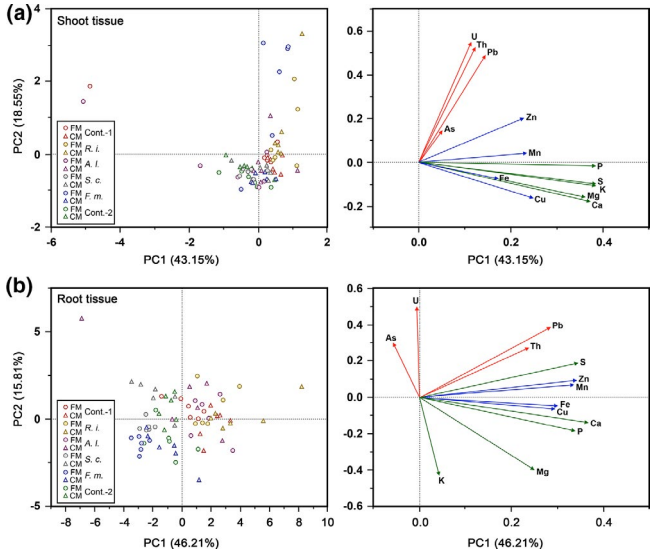

FIGURE 7 Principal component analysis (PCA) biplots (left) and loading plots (right) comparing element changes in *Plantago lanceolata* shoots (a) and roots (b) inoculated with *Rhizophagus irregularis* (*R.i.*) compared to the non-inoculated control (Cont.-1) after 8 weeks growth, and with *Acaulospora longula* (*A.l.*), *Scutellospora calospora* (*S.c.*), and *Funneliformis mosseae* (*F.m.*) compared to the non-inoculated control (Cont.-2) after 12 weeks growth. The 13 element variables include non-essential elements (U, Th, As and Pb) indicated by red loading vectors, essential macronutrients (Ca, Mg, K, P and S) indicated by green loading vectors, and essential micronutrients (Cu, Fe, Mn and Zn) indicated by blue loading vectors

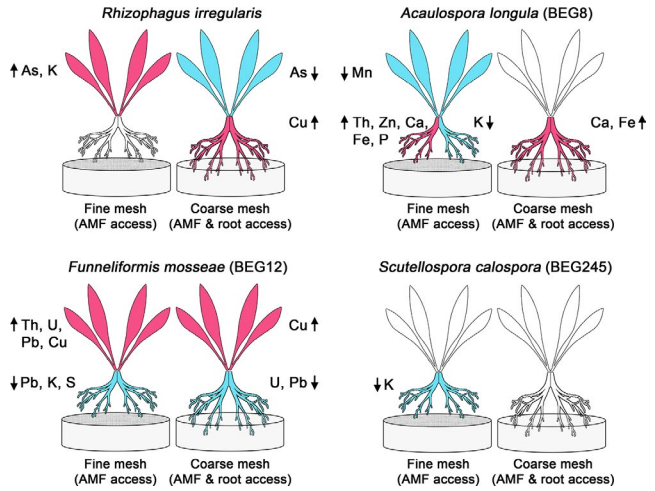

FIGURE 8 Summary of the element changes in *Plantago lanceolata* roots and shoots inoculated with *Rhizophagus irregularis*, *Acaulospora longula*, *Scutellospora calospora*, and *Funneliformis mosseae* either when only hyphae or when both roots and hyphae could access the South Terras soil. Increases (red) or decreases (blue) in element concentration relative to the non-inoculated control treatment are indicated

plant root cells are likely to be mediated via conserved cation transporters (Tamayo et al., 2014), some of which may be regulated by phosphate status (Xie et al., 2019), but none of these proteins have been previously associated with Th. Th uptake into plants, probably as Th^{4+}, is controlled by external phosphate availability such that low external phosphate concentration increases Th uptake into *Nicotiana tabacum* roots (Soudek et al., 2013). The presence of phosphate and Fe inhibits Th uptake, yet Ca facilitates Th uptake into *Brassica juncea* roots (Wang et al., 2015). Whether Th transfer into AMF shares similar characteristics is unknown.

4.2 | Plant responses to *R. irregularis* may be host specific

Many previous studies examining AMF mediated radionuclide and metal transfer used *R. irregularis*. In most cases, this fungal species is able to enhance root retention of elements and restrict shoot accumulation. For example, altered root:shoot ratio for U was observed in *Trifolium subterraneum*, *Medicago truncatula* and *Hordeum vulgare* inoculated with *R. irregularis* (Chen, Jakobsen, et al., 2005; Chen, Ross, et al., 2005; Rufyikiri et al., 2004), and the same response was seen for Th in mycorrhizal *M. truncatula* (Roos & Jakobsen, 2008). In contrast, *R. irregularis* inoculated *Lolium perenne* showed minimal change in root and shoot U concentration (Chen et al., 2008) while *R. irregularis* inoculated *P. vittata* showed increased shoot and root U concentration (Chen et al., 2006). The association of *R. irregularis* with *P. lanceolata* in our study had no significant influence on the accumulation of non-essential elements relative to the control, apart from some shoot As concentration changes (Figure 3). Thus, the diversity of responses to *R. irregularis* inoculation will depend on the host plant, which is in agreement with previous observations that behaviours vary with each fungus–plant combination (Helgason et al., 2002). However, a previous study found that *R. irregularis* inoculation of *P. lanceolata* led to significant reduction in root and shoot As and Pb, reduction in root P and increase in root Zn (Orłowska et al., 2012), in contrast with our observations. Differences in metal concentration and other abiotic characteristics of the source soil would likely explain the variation between studies. Another key difference is that while we grew *R. irregularis*-inoculated *P. lanceolata* for 8 weeks, Orłowska et al. (2012) grew their plants for 16 weeks, therefore temporal differences would likely be important. Finally, previous studies with *R. irregularis* have used strains other than DAOM197198, and therefore this could also explain the differences between them.

4.3 | No relationship between element partitioning and biomass changes

It was perhaps surprising that none of the fungal treatments gave a significant increase in root or shoot biomass (Figure 2). This is distinct from other similar studies; the two-compartment growth system used here was analogous to that used by Chen, Jakobsen, et al. (2005) who found significant increase in root and shoot biomass of *M. truncatula* by nearly twofold following *R. irregularis* inoculation.

Likewise, comparison of different fungal species including two *Rhizophagus* spp. and a *Funneliformis* sp. showed that all substantially increased *P. lanceolata* biomass (Orłowska et al., 2012). Instead we observed reductions in *P. lanceolata* root and shoot biomass for some AMF treatments (Figure 2). A negative impact on plant growth could be seen as a failure by a symbiont fungus to deliver a required nutrient to the plant and an excessive cost for the plant's carbon resources (Smith & Read, 2008). However, plant nutrient concentration was not inhibited in the treatments that showed a biomass decrease. A long-term experiment would be required for a more complete picture of the role of these cultures during the plant's life stages. There was also no correlation between reduced biomass and higher concentration of potentially toxic elements. Indeed increased Th, U and Pb concentration in the *F. mosseae* inoculated plants was coupled with a slight increase in shoot biomass.

5 | CONCLUSIONS

Understanding how different AMF species may influence the phytoaccumulation and aboveground partitioning of potentially toxic elements such as radionuclides has implications for phytoremediation applications and assessment of food chain transfer risk. Overall, these experiments have confirmed our hypothesis that AMF can significantly influence the transfer of non-essential elements into the host plant. Furthermore, they have highlighted the opposite roles of *A. longula* and *F. mosseae* in elemental uptake when associated with *P. lanceolata*. *F. mosseae* can transfer non-essential elements including ^{238}U, ^{232}Th and Pb to shoots whereas *A. longula* can concentrate elements including ^{232}Th, Ca, Fe and Zn into roots. Identifying AMF cultures that can mediate alteration to ^{232}Th is of interest and novel, as most previous AMF radionuclide studies have focused on U.

ACKNOWLEDGEMENTS

JRM was funded by a CONACyT PhD studentship from the Mexican government. Part of the study was funded by the NERC RATE (Radioactivity and the Environment) programme (grant number NE/L000547/1). We thank the Boconnoc Estate, Lostwithiel, Cornwall for allowing access to the South Terras site, and Prof Davey Jones (Bangor University) for permission to obtain soil from the Henfaes Research Centre, Bangor, Wales. We thank Paul Lythgoe (Manchester Analytical Geochemical Unit) for performing chemical analysis, Abby Ragazzon-Smith for technical support, Menghan Lu for soil preparation, James Dinsley for comments to the manuscript and Christopher Walker for advice and comments.

AUTHOR CONTRIBUTIONS

All authors designed the experiment, interpreted the data, and wrote the manuscript. JRM performed all experiments and data analysis.

ORCID

Jon K. Pittman https://orcid.org/0000-0001-7197-1494

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Rosas-Moreno J., Pittman J. K., & Robinson C. H. (2021). Specific arbuscular mycorrhizal fungal–plant interactions determine radionuclide and metal transfer into *Plantago lanceolata*. *Plants, People, Planet*, *3*(5), 667–678. https://doi.org/10.1002/ppp3.10185